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Tumor Necrosis Factor Alpha Promotes Replication and Pathogenicity of Rat Cytomegalovirus

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We investigated the role of tumor necrosis factor alpha (TNF-α) in the pathogenesis of rat cytomegalovirus (RCMV) infection. TNF-α levels found in the sera of radiation-immunosuppressed rats in the course of infection (>350 pg/ml) correlated with the development of RCMV disease. Administration of anti-TNF-α antibodies strongly reduced the severity of pneumonia and led to a reduction in virus titers. In immunocompetent rats, anti-TNF-α antibodies also significantly suppressed viral replication. Conversely, administration of TNF-α augmented RCMV replication and aggravated the disease signs. In vitro, TNF-α enhanced RCMV replication in the macrophage, whereas a reduction of viral replication was observed in fibroblasts, indicating that the effect on viral replication is cell type specific. Besides activation of viral replication and exacerbation of RCMV disease, TNF-α also favored lymphoid and hematopoietic tissue reconstitution after irradiation, which may contribute to antiviral resistance and survival. This finding demonstrates the protean nature of TNF-α, with both beneficial and adverse effects for the host. Our results suggest that TNF-α plays an important role in modulating the pathogenesis of RCMV infection.

Tumor necrosis factor (TNF) is an important mediator in inflammatory and immune reactions. Depending on the amount, time, and place of released TNF, this cytokine can have either beneficial or adverse effects on the course of an infection (for reviews, see references 3 and 39). Administration of TNF neutralizing antibodies during septic shock and cerebral malaria has shown to be protective (8, 37). However, its function in viral infections is largely unknown. Among its pleiotropic effects, TNF possesses a remarkable antiviral activity and the ability to kill virus-infected cells (18, 40). On the other hand, a central role for TNF in activation of virus replication and immunopathology was hypothesized after studies on the interaction of cytokines with human immunodeficiency virus replication (5, 16, 24).

Cytomegalovirus (CMV) is an important cause of morbidity and mortality in persons receiving immunosuppressive treatment, e.g., leukemia patients and allogenic bone marrow transplant recipients (12, 19). Moreover, CMV is the most common opportunistic viral pathogen in patients with AIDS (7, 17). Recently, increased serum TNF levels were detected in liver transplant patients who had developed a CMV infection (35); TNF-α mRNA was shown to be abundantly present in the colonic mucosa from AIDS patients with a CMV colitis (32). CMV infection of phorbol ester-differentiated promyelocytic ML-3 cells resulted in enhanced expression of TNF-α mRNA (4). Therefore an involvement of TNF in the pathogenesis of CMV disease may be assumed.

It is an objective of our studies to elucidate the pathogenetic role of cytokines in virus infections. For this purpose, we have established a rat infection model using an autochthonous CMV. The rat CMV (RCMV) resembles its human counterpart: it replicates poorly in the immunocompetent host but reaches high titers in organs of immunosuppressed animals, leading to characteristic CMV disease symptoms (2, 34). Pneumonia, cachexia, inhibition of hematopoiesis, and thrombocytopenia observed after CMV infection also occur upon administration of high doses of recombinant TNF in rats (36, 38). In this study, we describe the role of TNF-α in the pathogenesis of RCMV disease.

MATERIALS AND METHODS

Animals. Inbred specific-pathogen-free male brown Norway rats (90 to 110 g of body weight, 4 to 5 weeks of age) were obtained from the Experimental Animal Service, University of Limburg, Maastricht, The Netherlands. Animals were kept in filter top cages. The experimental protocols had been approved by the institutional Animal Welfare Committee.

Virus. Stocks of RCMV were prepared as 10% (wt/vol) homogenates of salivary gland tissue taken from brown Norway rats that had been inoculated 3 weeks previously with 10^4 PFU of RCMV via the intraperitoneal (i.p.) route. RCMV was passaged in rat embryo fibroblast (REF) cells. At maximal cytopathic effect, supernatants were harvested and centrifuged at 900 × *g* for 10 min. Virus was plaque titrated (2) and stored in aliquots at −70°C until use.

Assay for TNF. TNF activity in sera, tissue homogenates, and tissue culture supernatant was determined in a biological assay using WEHI-164 mouse fibrosarcoma cells (6). Cells were collected during the logarithmic growth phase and suspended at a concentration of 4×10^5/ml in RPMI 1640 medium containing 10% fetal calf serum (FCS). Fifty microliters of the suspension was added in triplicate to 50-μl volumes of the test sample in flat-bottom 96-well plates (Nunc, Breda, The Netherlands) and incubated at 37°C for 18 h. TNF specificity was established by incubation of the sample for 1 h at 37°C with either an excess of affinity-purified (recombinant murine TNF-α [rmTNF-α]–Sepharose) goat anti-TNF-α antibodies or a hamster monoclonal antibody (MAb) directed

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against murine TNF-α (Sanbio, Uden, The Netherlands) able to neutralize rat TNF-α (29). Cytotoxicity was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma). After addition of 20 μl of MTT (5 mg/ml in phosphate-buffered saline [PBS]) to each well and incubation at 37°C for 4 h, 100 μl of lysis buffer (20% [wt/vol] sodium dodecyl sulfate in 50% dimethyl formamide) was added; incubation was continued overnight at 37°C, after which time the A_{578} was read. Concentrations of TNF are given in picograms per milliliter, using rmTNF-α as a standard (specific activity of 75×10^6 U/mg of protein, as determined in L929 cells).

LPS assay. The lipopolysaccharide (LPS) content of samples was determined by the COA test (Chromogenix, Amsterdam, The Netherlands). The detection limit was 3 pg/ml.

Experimental design. One day after immunosuppressive whole-body irradiation (5 Gy, at 350 cGy/min), rats were infected via the i.p. route with 5×10^4 or 2×10^5 PFU of RCMV, as indicated in Results. On day 1 postinfection (p.i.), a group of animals was given an i.p. injection of a goat anti-recombinant rat TNF serum (3.5×10^4 neutralizing units per rat), while control rats received normal goat serum or PBS (all injections were of 200 μl per rat). To study the effect of exogenous TNF, rats were injected i.p. with rmTNF-α (1 μg/150 g of body weight in 200 μl of PBS containing 1% bovine serum albumin [BSA]) for 5 consecutive days. Orbital blood samples were allowed to clot at 4°C for 1 h, centrifuged, and kept at −20°C until use. Organs were removed on day 7 p.i. and homogenized (10% [wt/vol]) in Dulbecco's modified Eagle's medium containing 10% FCS (DMEM-FCS); supernatants after low-speed centrifugation were assayed for infectious virus.

Infectivity titration. Infectivity titers of RCMV were determined by using a plaque assay on REF cells as described previously (2). Plaques were counted 6 days p.i. after fixation of the monolayers with 10% formalin in PBS and staining with 1% aqueous methylene blue. Titers are expressed as PFU per gram of tissue.

Histology. Organ samples were fixed immediately after removal in liquid nitrogen and kept at −70°C until use or fixed in paraformaldehyde-lysine periodate and embedded in paraffin. Paraffin sections (5 μm) were deparaffinated and rehydrated in Tris-buffered saline (0.05 M Tris-HCl [pH 7.6]). The sections were stained with hematoxylin or incubated with 1% H_2O_2 in methanol for 15 min to block endogenous peroxidase activity and preincubated for 20 min at room temperature with normal goat serum (diluted 1/40) to reduce nonspecific binding. Cryostat sections (8 μm, cut at −30°C) were fixed for 10 min in acetone containing 0.02% H_2O_2. Sections were examined for the presence of viral antigen as described previously (34). Briefly, the slides were washed and incubated overnight at 4°C with a mixture of MAbs 8 and 35, directed against nuclear and cytoplasmic RCMV antigens, respectively, or goat anti-TNF-α antibody. All reagents were diluted in PBS containing 0.1% BSA and titrated to obtain optimal results. Subsequently, slides were washed three times for 5 min each time and incubated for 30 min at room temperature with the diluted secondary antibody conjugates (rabbit anti-mouse peroxidase conjugate; Sigma) containing 1% normal rat serum. All washes were in PBS. Peroxidase activity was visualized by using 0.003% H_2O_2 and 0.5% 3,3'-diaminobenzidine in 0.05 M Tris-HCl buffer (pH 8.3). The preparations were counterstained with hematoxylin, dehydrated, and mounted.

Cells. REF cells were prepared from 17-day-old rat embryos and grown in DMEM-FCS, penicillin (100 IU/ml), streptomycin (100 μg/ml), and amphotericin B (5 μg/ml). Cells were grown in plastic culture flasks (Nunc, Breda, The Netherlands) at 37°C in a humidified CO_2 incubator and used at the third passage. For isolation of spleen macrophages (spleen adherent cells), rats were sacrificed, their spleens were minced, the erythrocytes were lysed, and cells were seeded into 16-mm-diameter dishes of 24-well plates. After 1 h, nonadherent cells were removed and the remaining cells cultured for indicated times in DMEM-FCS. Adherent cells were identified as macrophages from their morphology and their nonspecific esterase activity (>95% positive).

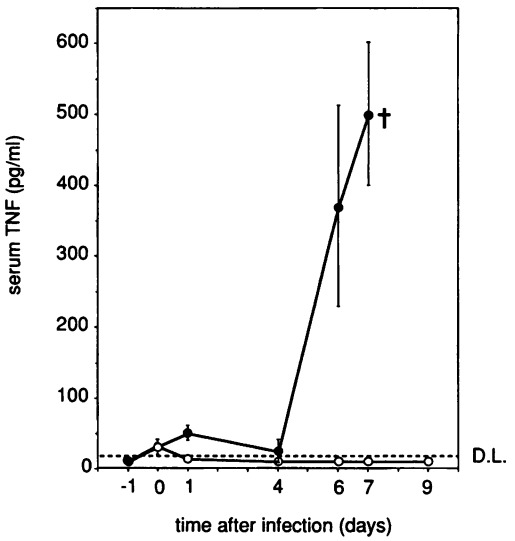

FIG. 1. TNF activity in sera of immunosuppressed RCMV-infected rats. The animals were subjected to whole-body irradiation and injected 1 day later with 2×10^5 PFU of RCMV (closed circles) or PBS (open circles) via the i.p. route. Data are expressed as means ± standard errors of the means (of eight animals). The dashed line indicates the detection limit (D.L.) for TNF, which was 10 pg/ml.

Antiviral assay. REF cells and spleen macrophages were treated with rmTNF-α (specific activity of 75×10^6 U/mg of protein, as determined in L929 cells) for 24 h and infected with RCMV at a multiplicity of infection as indicated. After 1 h, the inoculum was removed, and cells were washed and cultured in DMEM-FCS. Plaques and viral antigen-positive cells were counted at 3 days p.i. Cells were fixed with 70% ethanol (5 min at room temperature) and washed with PBS. Staining for RCMV antigen was performed as described for histology.

RESULTS

Detection of TNF in RCMV-infected rats. Radiation-immunosuppressed rats were infected with 2×10^5 PFU of RCMV, and their sera were tested for the presence of TNF bioactivity. As depicted in Fig. 1, high TNF concentrations (>350 pg/ml) were observed in all infected animals on days 6 and 7 p.i. Preincubation of the serum samples with goat antibodies or a hamster MAb directed against murine TNF-α completely neutralized TNF activity. Samples obtained on day 7 p.i. were devoid of potentially TNF-inducing gram-negative bacteria and LPS (<19 pg/ml of serum). Uninfected rats contained only low levels of TNF in their sera shortly after whole-body irradiation, which dropped to levels of <10 pg/ml within 24 h. The TNF levels in rats inoculated with heat-inactivated (30 min, 56°C) virus were indistinguishable from those of uninfected control animals (data not shown).

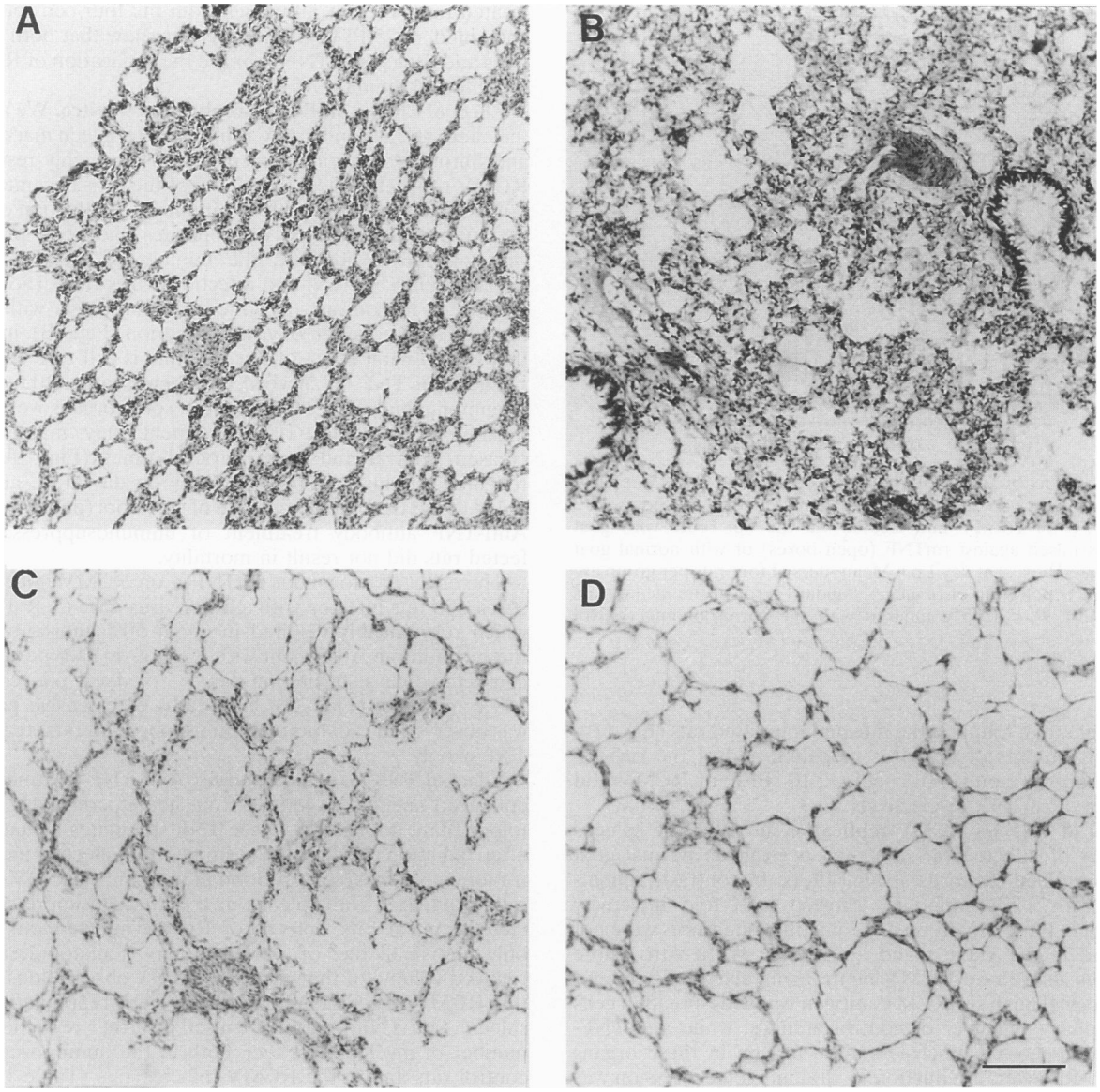

FIG. 2. Effects of anti-TNF antibodies and rmTNF on pneumonia in immunosuppressed RCMV-infected rats. Shown are representative hematoxylin-eosin-stained sections of the lungs 7 days p.i. from RCMV-infected (A to C) and control (D) rats which had received normal goat serum (A and D), rmTNF (B), or goat anti-TNF antibodies (C). Bar, 160 μm.

The increase in serum TNF levels correlated with the development of CMV lesions. On day 7 p.i. but not on day 3, disseminated intravascular coagulation accompanied by multiple hemorrhagic foci was detected on the serosal surfaces of the intestine and liver. At that time pneumonia was seen, and the lungs showed punctate areas of hemorrhage, edema, and infiltration with monocytes and some polymorphonuclear leukocytes. The infected rats succumbed within 8 to 12 days p.i., while no mortality was observed over a period of 30 days in the irradiated control animals.

In immunocompetent rats, low serum levels of TNF-α (40 to 85 pg/ml) were found on days 3 and 5 p.i. The bioactivity could be ascribed to TNF-α, since it was abolished in the presence of antiserum to recombinant rat TNF-α.

Effects of TNF on the pathology of RCMV disease. To determine the role of TNF-α in RCMV disease, immunosuppressed rats were treated on day 1 p.i. with an anti-TNF-α goat serum. After this treatment, TNF could no longer be detected in sera assayed on day 7 p.i.; also, these sera were capable of neutralizing rmTNF in vitro. The anti-TNF antibodies had a conspicuous effect on lung pathology: while infected rats displayed extensive pneumonitis (Fig. 2A), lung tissue of the anti-TNF-treated animals appeared intact and only sporadically showed small foci of inflammation (Fig. 2C). Furthermore, in treated animals, less thrombosis and ecchymoses in the liver and kidneys were observed; however, no significant differences in body weight were found between the infected animals treated with anti-TNF or normal goat serum (data not shown).

When infected animals were additionally treated with TNF (1 μg of rmTNF per day for 5 consecutive days starting on day 1 p.i.), histological examination revealed a more pronounced

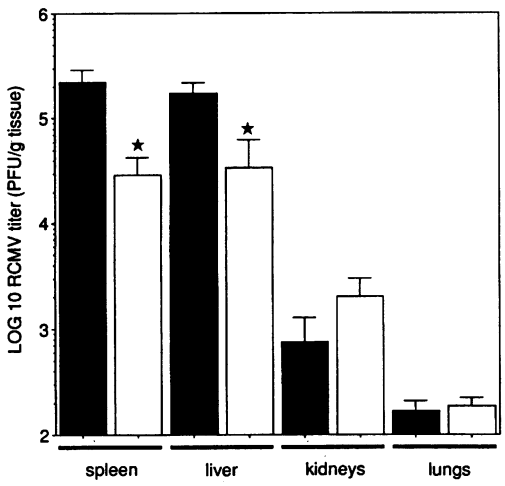

FIG. 3. Effect of anti-TNF antibody treatment on RCMV replication in vivo. Rats were infected 1 day after immunosuppression with 2 $\times$ 10^5 PFU of RCMV and treated via the i.p. route with goat antibodies raised against rmTNF (open boxes) or with normal goat serum (closed boxes) at day 1 p.i. Mean titers of four rats per group are given as PFU per gram of tissue $\pm$ standard error of the mean; stars indicate EM; $P < 0.05$ compared with the corresponding control organ.

pneumonia than found in the infected untreated rats (Fig. 2B). The LPS contents of the virus inoculum and of the rmTNF preparation were only 0.012 ng/5 $\times$ 10^4 PFU of RCMV and 0.2 ng/μg of rmTNF, respectively.

Effects of TNF on RCMV replication in vivo. The spleens and livers of infected rats that had been given normal goat serum contained the virus at high titers. In contrast, animals treated with TNF antibodies showed a 10-fold infectivity reduction in these organs on day 7 p.i.; the differences were not significant in the kidneys and lungs (Fig. 3). In vitro, these antibodies showed no RCMV neutralizing activity. By immunohistological analysis, RCMV antigen was widespread in cells of the spleen and liver of control animals, while anti-TNF-treated rats showed much less viral antigen in these organs (Fig. 4). Moreover, a reduction in viral antigen expression was observed also in the lung alveoli. In the kidneys of control rats, viral antigen occurred predominantly in the glomeruli, whereas in antibody-treated rats, predominantly capsular serosal cells were antigen positive (not shown). Thus, redistribution of virus within or between some organs may have occurred.

Immunocompetent rats infected i.p. with 10^6 PFU of RCMV exhibited no signs of disease. Frozen spleen sections obtained at different time points after infection and stained with anti-RCMV MAbs revealed a maximum number of antigen-containing cells on day 5 p.i. As shown in Fig. 5, TNF-α neutralizing antibodies produced a 10-fold reduction of the number of RCMV-infected cells in the spleen in comparison with control rats receiving an irrelevant goat antiserum. In contrast, administration of anti-asialo glycoprotein antibodies resulted in a 10-fold increase in the numbers of virus-infected cells, indicating that natural killer cells control replication in the spleen.

The effects of exogenous TNF-α (1 μg of rmTNF per day for 5 consecutive days starting on day 1 p.i.) were studied in immunosuppressed rats infected with a lower virus dose (5 $\times$ 10^4 PFU). On day 7 p.i., infectivity titers of 1.1 $\times$ 10^3 $\pm$ 0.5 $\times$ 10^3 and 1.3 $\times$ 10^3 $\pm$ 0.4 $\times$ 10^3 PFU/g of spleen and liver tissue, respectively, were determined in five of eight TNF-treated rats, whereas all tissues from the four control animals contained <125 PFU/g. These results show that both endogenous and exogenous TNF enhance the replication of RCMV in the infected animal.

Effect of TNF-α on RCMV replication in vitro. We analyzed the effect of TNF on RCMV replication in spleen macrophages and fibroblasts. In vitro, macrophages are highly resistant to RCMV replication: less then 0.1% of the cells became antigen positive after infection with a multiplicity of infection of 3 (Fig. 6A). When the cultured macrophages had been pretreated with rmTNF-α, however, the number of RCMV antigen-positive cells (Fig. 6A) and infectious virus titers (not shown) increased. In contrast, treatment of fibroblasts with TNF-α resulted in a reduction of viral replication (Fig. 6B), indicating that the antiviral effect of this cytokine is cell type specific.

Effects of TNF on survival of infected rats. Although both pneumonia and the virus content in some organs were unmistakably reduced, anti-TNF treatment only marginally increased survival and mean survival times (Fig. 7A). Also, repeated administration of antibody on days 1, 3, and 5 p.i. failed to increase the percentage of survivors (data not shown). Anti-TNF antibody treatment of immunosuppressed uninfected rats did not result in mortality.

The effect of exogenous rmTNF-α on RCMV infection was studied in rats infected with a lower virus dose (5 $\times$ 10^4 PFU) which reproducibly resulted in about 50% mortality. Under these conditions, treatment with 1 μg of rmTNF per day for 5 consecutive days starting on day -3 or day 1 p.i. resulted in 100% mortality (Fig. 7B). No deaths or extensive pathology was observed in radiation-immunosuppressed rats treated with TNF-α only.

Effect of TNF on hematopoiesis. Anti-TNF treatment led to enhanced anemia, as judged from hematocrit levels and histology (data not shown). Since TNF stimulates hematopoiesis after radiation damage, we examined the effect of its neutralization on splenic reconstitution in irradiated, RCMV-infected rats. Compared with spleens of the immunosuppressed uninfected control rats, spleens of RCMV-infected rats showed only few small foci of extramedullary hematopoiesis and a reduced volume of the white pulp; these observations indicate that RCMV infection inhibits myelopoietic repopulation in the spleen. Anti-TNF treatment caused a further reduction of the number of myelopoietic foci, both in the immunosuppressed control rats and in the RCMV-infected rats (Table 1). These results demonstrate that besides activation of viral replication and exacerbation of CMV disease, TNF also favors lymphoid and hematopoietic tissue reconstitution after irradiation, which may contribute to antiviral resistance and survival.

DISCUSSION

Induction of TNF appears to play a central role in the pathogenesis of diverse infectious diseases (8, 37, 39). Several viruses are known to induce TNF expression in vitro (1, 4, 40), but only a few in vivo studies have been dedicated to this cytokine. In this report, we demonstrate that TNF-α plays a role in RCMV-induced pathology, as a cytotoxic molecule and as a promoter of virus replication.

The pathology accompanying CMV disease includes pneumonia, anemia, thrombocytopenia, and blood coagulation disorders; all these have also been found during septic shock, a clinical situation mediated by the toxic action of pro-inflammatory cytokines, in particular TNF. Treatment of rats with recombinant TNF results in pneumonia and anemia (36, 38). The cytokine may stimulate hematopoiesis and subsequently shift it from the erythroid to the macrophage-granulocyte

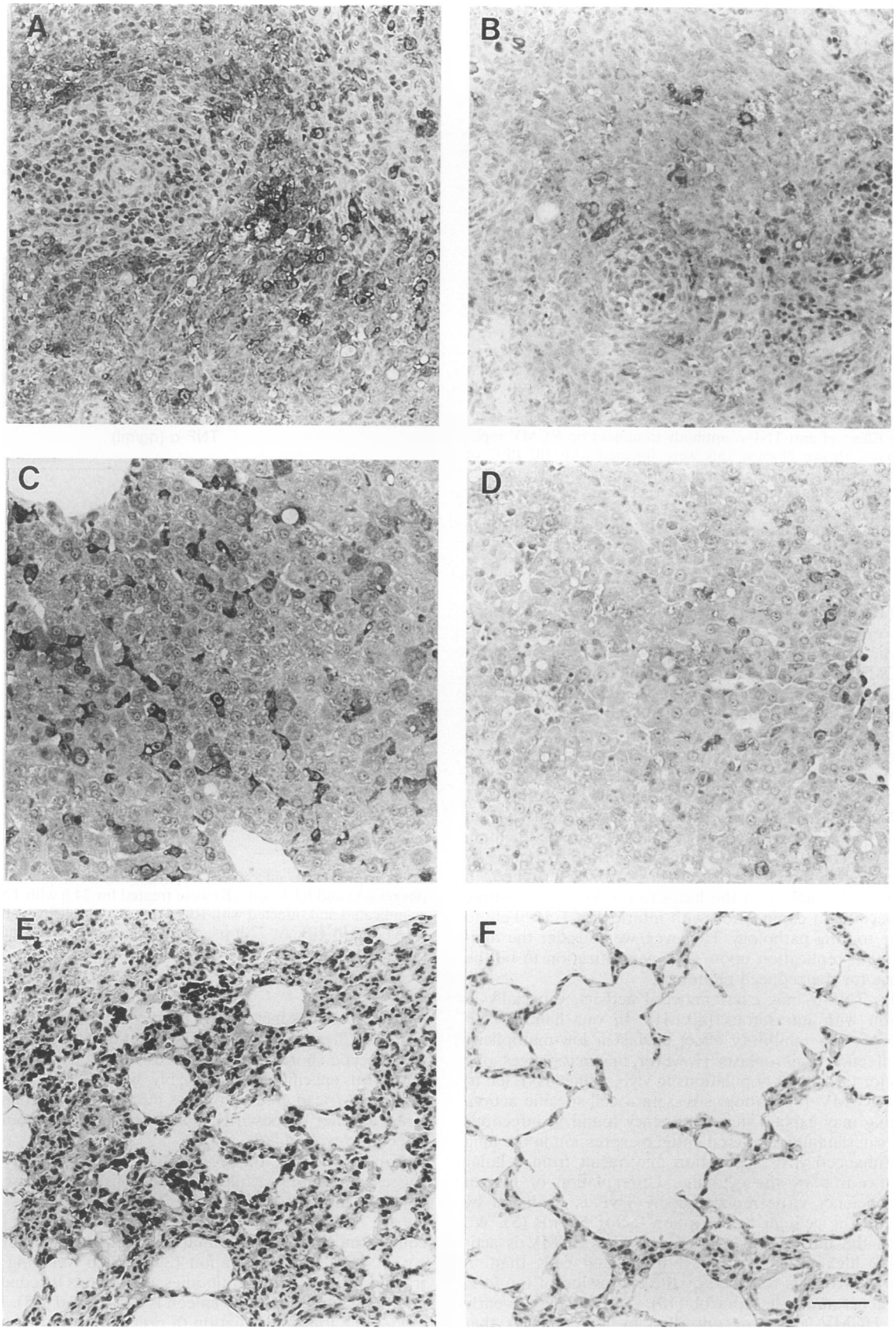

FIG. 4. Immunohistological analysis of the effects of anti-TNF antibodies on RCMV replication in vivo. Representative anti-CMV-stained sections of the spleen (A and B), liver (C and D), and lungs (E and F) 7 days p.i. from RCMV-infected rats which had received normal goat serum (A, C, and E) or goat anti-TNF antibodies (B, D, and F) are shown. Bar, 62.5 μm.

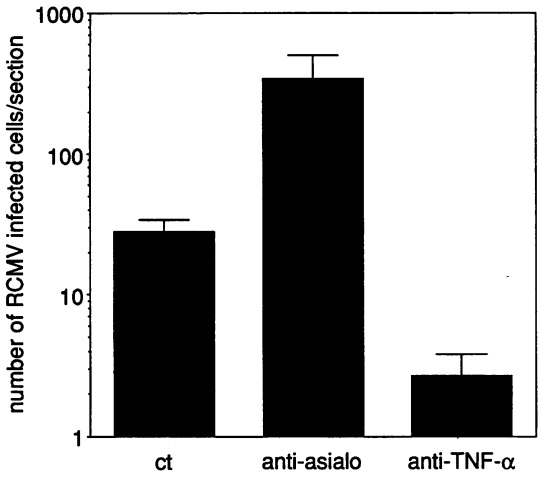

FIG. 5. Effect of anti-TNF-α antibody treatment on RCMV replication in vivo. Brown Norway rats were infected with 10^6 PFU of RCMV and treated via the i.p. route with anti-asialo glycoprotein antibody, goat antibodies raised against rmTNF-α, or normal goat serum (ct). Viral replication in the spleen at day 4 p.i. is given as the mean number (± standard error) of RCMV-infected cells per spleen section of four rats per group.

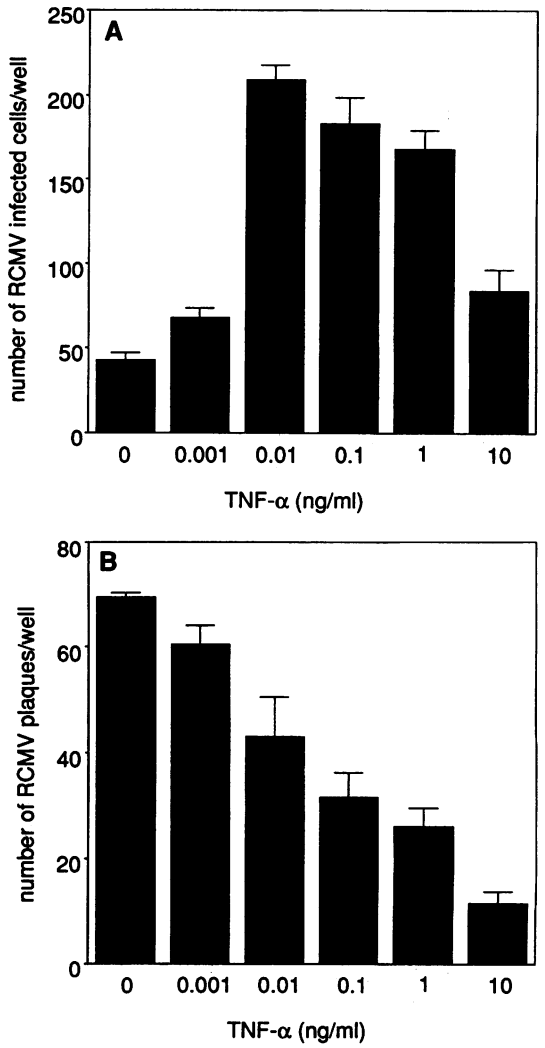

FIG. 6. In vitro effect of TNF-α on RCMV replication. Macrophages (A) and REF cells (B) were treated for 24 h with TNF-α doses as indicated and infected with RCMV at a multiplicity of infection of 3 (A) or 0.01 (B). At 72 h p.i., the numbers of RCMV-infected cells (A) or plaques (B) were determined.

lineage (13). This happens under circumstances in which enhanced defenses are required, which causes a dyserythropoietic anemic state characterized by a low erythrocyte mass. In human CMV (HCMV) disease, especially in infected newborns, severe anemia and disturbances of the hematopoietic system are believed to constitute lethal features (23).

Our finding of a mitigated pneumonia in anti-TNF-treated animals may be of therapeutic relevance. Interstitial pneumonia is a major complication in HCMV-infected immunosuppressed patients (12). Grundy et al. (9) hypothesized that CMV pathology is caused by cytotoxic mediators released from infiltrating T cells upon recognition of virus-infected cells in the lung. Whether the TNF release seen in our system is by lymphoid or virus-infected cells remains to be determined. In view of the vulnerability of the lungs to cytokines, circulating TNF, especially in conjunction with interleukin-1, is likely to contribute to lung pathology. However, we consider the inhibition of viral replication upon TNF neutralization to be the main cause for the reduced pathology.

In vitro, TNF-α may exert antiviral activity, especially in conjunction with interferons (40, 41). In our hands, TNF displayed also an inhibitory effect against a low-multiplicity RCMV infection in fibroblasts. However, in macrophages, one of the major target cell populations in vivo, added TNF led to enhanced RCMV replication, stressing a cell-specific action. This finding may explain the discrepancy found in infectious virus titers in the lungs and viral antigen expression in the lung alveoli. Enhanced virus replication may result from cellular activation caused by the cytokine. Upregulation of human immunodeficiency virus replication by TNF is mediated by induction of the cellular transcription factor NF-κB (5). We found that the immediate-early promoter of HCMV is activated to a higher extent in TNF-pretreated cells than in untreated controls, as reflected by the expression of the *lacZ* reporter gene under its control (10). The immediate-early genes of HCMV are also controlled by an enhancer that contains binding sites for NF-κB (28).

How can the results be reconciled with the antiviral effects attributed to TNF? It should be noticed that TNF-mediated protective effects have been observed in experimental vaccinia virus and herpes simplex virus infections (25, 27) but not in lymphocytic choriomeningitis virus infection (14, 26), indicating a virus specificity. Remarkably, when TNF-α was administered to African green monkeys infected with simian varicella virus, another herpesvirus, again a more severe course of the infection resulted (33).

Conservation of the TNF gene in mammalian evolution suggests that this cytokine is responsible for useful, even essential functions, apart from its pathogenic roles discussed above. At low doses, TNF may recruit cells from the blood compartment and exert multiple immunoregulatory effects, e.g., T- and B-cell activation (39). When weanling mice are treated with anti-TNF antibodies, the thymus is reduced in size and the white pulp in the spleen is diminished (15). In our rat model, the ionizing radiation (a precondition to allow RCMV replication) temporarily destroys lymphoid and hematopoietic cells; it transiently induces circulating TNF, which probably acts as a radioprotector (11). The cytokine shows radioprotec-

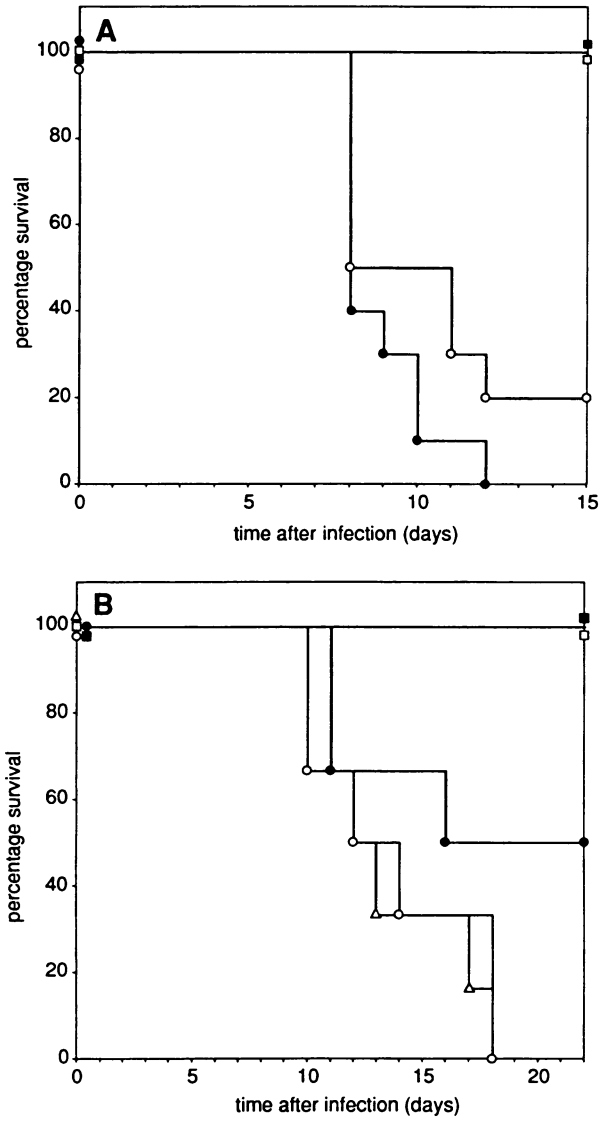

FIG. 7. Effects of anti-TNF antibodies and rmTNF on the survival of radiation-immunosuppressed, RCMV-infected rats. (A) The animals (10 per group) were immunosuppressed by whole-body irradiation, injected 1 day later with 2×10^5 PFU of RCMV (circles) or PBS (squares), and treated with goat antibodies raised against rmTNF (open symbols) or normal goat serum (closed symbols) given via the i.p. route. (B) The animals (six per group) were injected 1 day after immunosuppression with 5×10^4 PFU RCMV (circles and triangles) or PBS (squares) and treated with rmTNF-α (open symbols) for 5 consecutive days starting at day −3 (triangles) or day 1 (circles and squares) or with PBS (closed symbols) given via the i.p. route.

tive effects in animals given sublethal whole-body irradiation by allowing earlier hematopoietic recovery (31), probably via the secretion of hematopoietic growth factors (20). This is consistent with our finding that infected anti-TNF-treated rats show a further reduction of hematopoiesis in their spleens (Table 1) and may explain the failure of TNF neutralization to protect rats from death despite a reduction in viral titers and diminished pathology. Increased radiosensitivity has also been observed in sublethally irradiated mice receiving anti-TNF antibodies (22). Although accompanied by high TNF concentrations, RCMV infection manifestly suppressed hematopoiesis in the spleen. Immunosuppressive mechanisms induced by RCMV which are not counteracted by TNF may therefore be operative. Indirect inhibition of hematopoiesis has been postulated as the primary cause of death in immunocompromised mice suffering from murine CMV disease (21). Some HCMV isolates also affect myelopoiesis directly by infecting myeloid progenitor cells, whereas others infect bone marrow stromal cells (30).

TABLE 1. Effect of anti-TNF antibodies on hematopoietic recovery

Treatment	No. of hematopoietic foci/spleen section[a]
PBS	87 ± 22
PBS + anti-TNF	29 ± 10
RCMV	23 ± 8
RCMV + anti-TNF	5 ± 3

[a] On day 8 postirradiation (n = 4). Treatment and interventions were conducted as described in the legend to Fig. 7A.

In conclusion, TNF produced during RCMV infection plays an important role in the enhancement of virus replication and pathology in immunosuppressed rats. Our data demonstrate the protean nature of TNF, with both beneficial and adverse effects for the host. Since both TNF and CMV have been identified as important factors in the progression of AIDS, inhibition of the expression or action of this cytokine may be beneficial in downregulating both viruses and the concomitant pathology.

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